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Published in:
Tetrahedron

DOI:
10.1016/j.tet.2017.06.049

Publication date:
2017

Tetrahedron 73 (2017) 4837–4848

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Perspectives

Designing dynamic functional molecular systems

Anouk S. Lubbe [1], Thomas van Leeuwen [1], Sander J. Wezenberg**, Ben L. Feringa*

Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 19 May 2017
Accepted 25 June 2017
Available online 2 July 2017

Keywords:
Self-assembly
Molecular switches
Molecular motors
Reaction networks
Biohybrid systems

ABSTRACT

The design and construction of dynamic functional molecular systems, which mimic some of the properties of living systems, pose a huge contemporary challenge. Recent developments in supramolecular self-assembly, molecular switches, motors and machines, and chemical reaction networks, offer an excellent basis for integrating dynamic properties in molecular systems. In this perspective, we discuss different approaches towards dynamic functional molecular systems covering areas such as translated motion, dissipative self-assembly, self-regulation and biohybrid systems. The selected examples illustrate the level of control and complexity that can be achieved at present in this rapidly growing and exciting field of research.

Contents

1. Introduction

A distinctive feature of synthetic chemistry is its amazing creating power as is evident from the wealth of molecules and materials that enable proper functioning of our modern society. From drugs to dyes, cables and cars to displays and detergents, it is the creativity in designing structure and function along length scales, ranging from small molecules to mesoscopic and macromolecular materials, where chemistry shows its nearly unlimited opportunities for exploration. The total synthesis of many of the most complex natural products is a clear testimony of the often ingenious way synthetic chemists have mastered chemical reactivity, functionality and covalent assembly of complex structures. With the advent of supramolecular chemistry, the stage is set for non-covalent assembly and molecular recognition processes far beyond the level of the individual molecules reminiscent to many phenomena in biological systems. In recent years a new dimension has been added with the introduction of dynamic covalent chemistry providing a stepping stone to adaptive and potentially evolutionary behavior.

A closer look at the molecules of life itself immediately leads to the realization that biomolecules do not function in isolation but are usually part of complex molecular systems that operate in a highly dynamic manner. Membrane transport, the process of vision, muscle movement, ribosomal peptide synthesis or bacterial flagellar rotation are just a few examples of the "machinery of life"[1] and the delicate interplay of complex biomolecular assembly and specific well controlled dynamic processes that ultimately allow a

* Corresponding author.
** Corresponding author.
E-mail addresses: s.j.wezenberg@rug.nl (S.J. Wezenberg), b.l.feringa@rug.nl (B.L. Feringa).
[1] Equal contributions.

http://dx.doi.org/10.1016/j.tet.2017.06.049
0040-4020/

specific biological function. Beyond the mere "synthesis for function" it is the design of dynamic functional molecular systems that offers great challenges for contemporary chemistry and molecular nanoscience. The recent progress in the development and application of molecular switches, motors and machines, provides a superb basis for the exploration of dynamic properties in molecular systems.

Moving from molecules to dynamic molecular systems, it is evident that the emphasis in the chemical design strategy shifts to complex and multicomponent molecular assemblies and responsive behavior. Besides the obvious precise design of molecular structure and its synthesis, control over organization, specific functions and/or tasks, the multi-molecular ensemble, interface phenomena and hierarchical levels, are some of the main aspects to deal with. Design, synthesis and reactivity are cornerstones in the approach but integration of structure and function, control of assembly and intrinsic dynamic properties are equally important. As the ultimate goal is dynamic and responsive function, it is essential to consider from the onset questions associated with triggering, addressing and sensing. Here the molecular designer can exploit a wide variety of chemical and physical triggers including pH, ion binding, redox, chemical conversion and light. Numerous opportunities arise when the suite of chemical catalysis methodology is to be exploited, i.e. coupling chemical conversions to control of dynamic functions. It should be reminded that biomolecular motor-driven mechanical processes in Nature are almost exclusively governed by catalytic conversions of chemical fuels (e.g. ATPase rotary motor). The study of dynamic behavior allows also the development of molecular systems that operate far from thermodynamic equilibrium akin of many natural processes. Other important challenges associated with dynamic molecular systems are cooperative or collective behavior, amplification of motion along many length scales (i.e. molecular, supramolecular, mesoscopic, microscopic, macroscopic) and interfacing to the micro-macro-worlds including control of hard-soft interfaces. Once the design principles are established and control over dynamic functions is achieved, the stage is set for autonomous processes as well as emerging and adaptive behavior. Signal transduction, molecular information processing, feedback mechanism and self-repair mechanisms are all major challenges ahead. Despite the complexity of the envisioned systems and the intricate interplay of multiple functions, the prospects in a more distant future for soft robotics, smart materials and numerous biomedical applications are particularly bright.

Here we discuss approaches towards dynamic functional systems and provide a perspective in areas ranging from molecular motion, responsive materials, dissipative and self-regulatory systems to dynamic bio-hybrid systems. The examples presented are not exhaustive but an illustration of the level of control of complex functions and dynamic properties that can be achieved in this young but rapidly emerging field.

2. From molecular to macroscopic motion

Among the most fascinating dynamic functional molecular systems are those in which motion at the molecular level translates into movement at the macroscopic scale. Initially, the driving force behind this research objective was to prove that molecular switches and motors can perform work.[2] However, because of the rapid progress in this field, functional applications of these systems are now becoming within reach. For example, in soft robotics since soft matter is the most frequently used material in this research.

The group of Ikeda showed that the photoinduced structural changes in azobenzene switches can be harnessed to achieve macroscopic motion, i.e. the directed bending of a thin polymer film.[3] The thin film was prepared by co-polymerization of azobenzene-containing liquid crystalline monomers with a diacrylate crosslinker affording a liquid crystalline film consisting of small domains. Each domain is composed of many azobenzene units, which are all aligned in the same direction, although on average, for all the domains, the alignment is randomly distributed. Irradiation with linearly polarized light, led to the *trans*-to-*cis* isomerization of only the azobenzene moieties aligned with the light's polarization direction. This isomerization resulted in a decrease in size of the liquid crystalline domains and hence, a contraction of the volume at the illuminated surface, which consequently led to bending of the film. The polymer film could be used as a transmission belt to power a rotary motor with light (Fig. 1a).[4] This example beautifully illustrates how motion at the molecular scale can induce macroscopic movement and moreover, how azobenzene photoswitches can be used to convert light energy into mechanical work.

Inspired by this work and also related studies conducted by Broer and co-workers,[5,6] the group of Katsonis took the next step in the development of these materials.[7] The addition of a small amount of chiral dopant to the azobenzene containing liquid crystalline matrix afforded thin films with extraordinary properties. Depending on the angle at which these films were cut, ribbon-like structures with either left or right handed helicity and varying pitch were obtained. The responsive behavior of these ribbons upon irradiation depended on their morphology: They either exhibited a winding or an unwinding motion, or displayed an inversion of their handedness. The group of Katsonis nicely demonstrated how this feature could be used to construct a functional device.[7] A ribbon with mixed helicities, where one half contracts upon irradiation while the other part expands, was able to perform work in the form of moving two magnets (Fig. 1b).

Our group presented a different approach to amplify molecular motion using the unique properties of liquid crystalline materials.[8] Instead of covalent functionalization, the doping of a nematic liquid crystal with small quantities of enantiopure molecular motor, resulting in the formation of a cholesteric phase, proved to be sufficient to control the liquid crystal properties. When the liquid crystal was placed on top of a glass slide covered with a unidirectionally aligned polyimide layer, photoisomerization of the chiral motor dopant led to a rotational reorganization of the cholesteric texture, which could be used to power the rotation of a microsized glass rod (Fig. 1c).

In addition to the use of light, chemical stimuli can be used to achieve macroscopic mechanical work via molecular motion. Skeletal muscles are a good example of how chemically-induced molecular motion can be harnessed to achieve function. Sauvage and co-workers conducted pioneering work on mimicking the action of muscles in a fully synthetic system.[9] In his daisy-chain rotaxane based system, the addition of Cu^{+} and Zn^{2+} ions led to an extending and contracting motion. Stoddart et al. demonstrated how this type of motion, in a rotaxane assembled on gold, induced by chemical oxidation and reduction, could be used to enable bending and straightening of a cantilever (Fig. 1d).[10]

One of the benefits of light-driven rotary molecular motors compared to bistable switches (e.g. azobenzene) is that they exhibit repetitive unidirectional motion. This feature is highly beneficial when continuous molecular motion is desired, for example, in the system developed by the group of Giuseppone who integrated a light-driven molecular motor into a polymeric PEG gel via a quadruple Click reaction (Fig. 2a).[11] The rotation of the motor led to the braiding of the polymer chains, resulting in gel shrinkage. In this way, light energy could be stored into the gel as potential energy with an efficiency of about 0.15%. However, it was impossible to harvest this energy as the process of braiding the polymeric

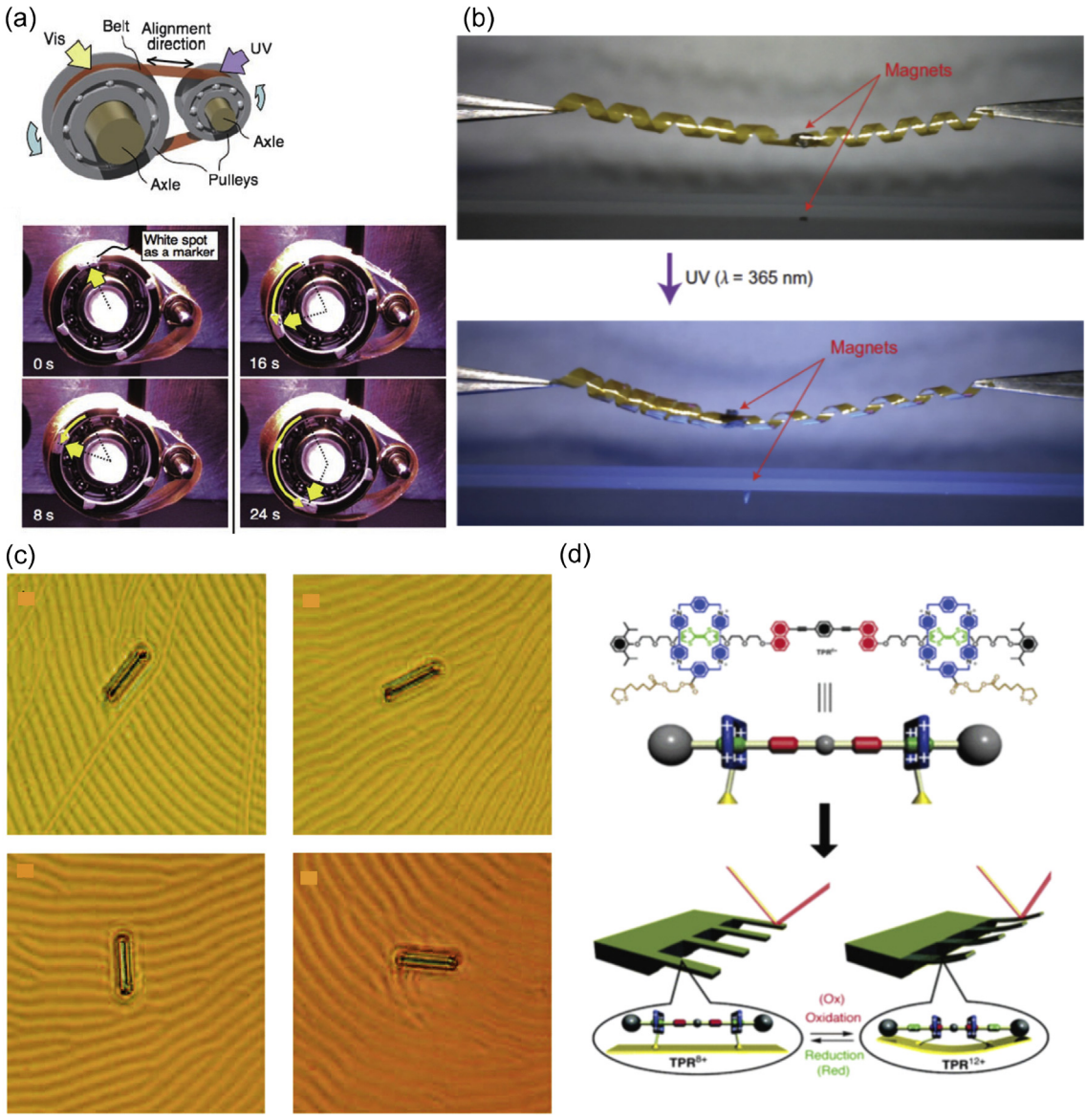

Fig. 1. a) Transmission of a rotary motor using a photoresponsive liquid crystalline film. Adapted with permission from Ref.[4], John Wiley & Sons, 2008. b) Movement of magnets induced by the coiling and uncoiling of a liquid crystal ribbon. Adapted with permission from Ref.[7], Nature Publishing Group, 2014. c) Rotation of a glass rod on a liquid crystal surface, induced by the rotary motion of a molecular motor. Adapted with permission from Ref.[8], Nature Publishing Group, 2006. d) Reversible bending of a cantilever by extension and contraction of a rotaxane. Adapted with permission from Ref.[10], AIP Publishing LLC, 2004.

chains was irreversible. A recent improvement on their design resolves this issue.[12] The incorporation of diarylethene switches, which can unbraid the polymer chains, made it possible to control the shrinkage and expansion of the gel using different irradiation wavelengths (Fig. 2b). When both UV and visible light are used with varying intensities, the motors braid the polymeric chains, while the diarylethene switches allow for unwinding of the polymer. If the intensities of the light sources are chosen in such a way that the speed of braiding and unbraiding is equal, then an out-of-equilibrium steady state can be achieved. It is thus nicely demonstrated how precise design at the molecular level can allow for the construction of more complex systems able to convert light energy into potential and mechanical energy. Besides liquid crystalline materials and gels, other materials such as crystals of diarylethene switches have been used to convert molecular motion into motion at larger length scales.[13]

As an alternative to incorporating photoswitches and motors into soft materials, surface functionalization can be used to translate molecular motion to larger length scales.[14,15] Leigh et al. developed an elegant system in which switchable wetting properties of surfaces are used to achieve directed macroscopic motion (Fig. 3a).[16] Their design consisted of a self-assembled monolayer of 11-mercaptoundecanoic acid on gold, which could be functionalized with rotaxanes via physisorption. The macrocycle in these rotaxanes could be shuttled between two stations *via* photoisomerization of a fumaride group. During this shuttling process, a perfluoroalkane moiety was either exposed or covered, which had significant impact on the polarophilicity of the surface. When a droplet of diiodomethane was placed on an inclined (12°) functionalized mica surface, and the front of the droplet was irradiated, an uphill movement was observed, which is due to a decrease in contact angle at the illuminated part of the surface.

In a different approach, our group assembled rotary motors in an altitudinal orientation on a gold surface using a tripodal

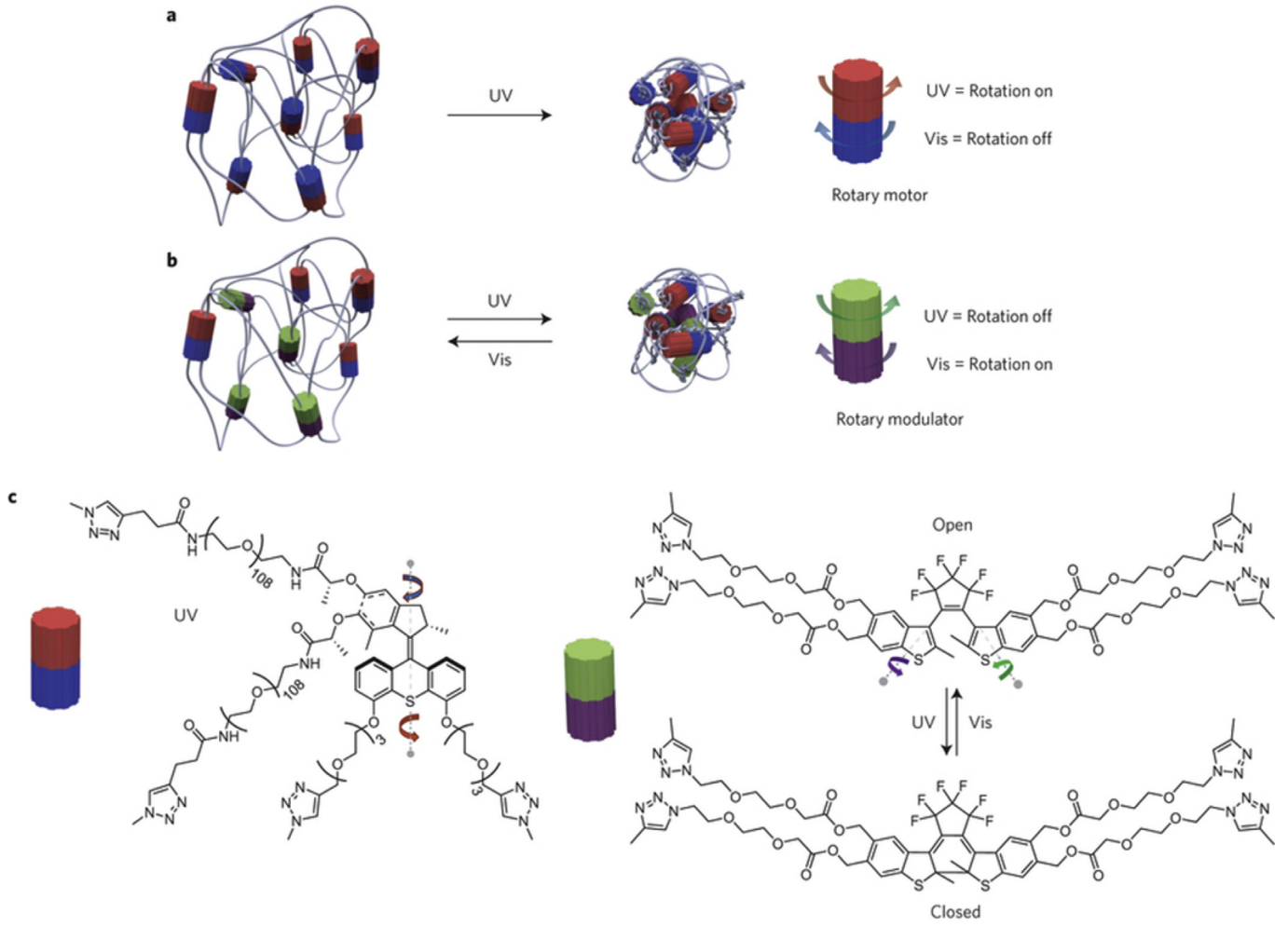

Fig. 2. a) The contraction of a gel due to the continuous rotation of molecular motors. b) The braiding and unwinding of polymeric network using molecular motors and diarylethenes. c) The components used in the responsive gels developed by Giuseppone. Adapted with permission from Ref.[12], Nature Publishing Group, 2015.

anchoring group (Fig. 3b). During the rotation of the motor, a perfluoroalkyl group is either exposed or concealed, which changes the wettability and thickness of the monolayer.

Next to systems that require light as a trigger, several systems have been developed which use the reversible desorption and adsorption of water as a stimulus for macroscopic motion.[18–21] Aida and co-workers found that a highly oriented film of carbon nitride polymer, prepared *via* vapor deposition polymerization, had remarkable properties (Fig. 4).[22] These films curled and straightened in response to small changes in ambient humidity. At constant humidity, deviations in temperature affected the desorption and adsorption of water which in turn resulted in the movement of the film. In addition, desorption of water could be achieved by irradiation. The films were able to bend in response to light and straighten again for more than 10.000 cycles, without deterioration of the sample. Another fascinating property of these films was that they were able to jump from a surface (up to 10 mm). As only small fluctuations in humidity or temperature are required to induce motion, these films are extremely efficient at harvesting energy from the environment to perform work.

3. Dissipative out-of-equilibrium assemblies

Inspired by nature, where many biological functions are executed by dissipative self-assemblies (e.g. tubulin assembly), it is now recognized that the design of synthetic assemblies with similar properties represents a promising way toward the development of dynamic functional molecular systems.[23,24] One of the crucial differences of dissipative self-assembly, compared to thermodynamically downhill self-assembly, is adaptability.[25] While equilibrium self-assembly represents a static situation, dissipative self-assemblies are dynamic in nature and need a continuous input of energy to sustain themselves.

Klajn, Grzybowski and co-workers could achieve the dissipative self-assembly of gold nanoparticles in organogel films using light.[26] The gold nanoparticles were decorated by azobenzene functionalized ligands and photoinduced formation of the polar *cis* azobenzene causes nanoparticle aggregation as a result of favorable dipole-dipole interactions. Here the assembly can be considered dissipative, because thermal relaxation to the thermodynamically favored *trans* azobenzene isomer would cause disassembly and thus, light energy is required to maintain the assembled structure (Fig. 5a). The films, in which the nanoparticles were dispersed, exhibited a color change upon exposure to UV light, which allowed for writing images using a mask. These images erase themselves over time due to the transient nature of the nanoparticle aggregates. In a later stage, Klajn and co-workers demonstrated other possible applications of these self-assemblies.[27] Solute molecules can get trapped inside the cavities of the aggregates and it was found that these cavities can function as reactors, able to accelerate

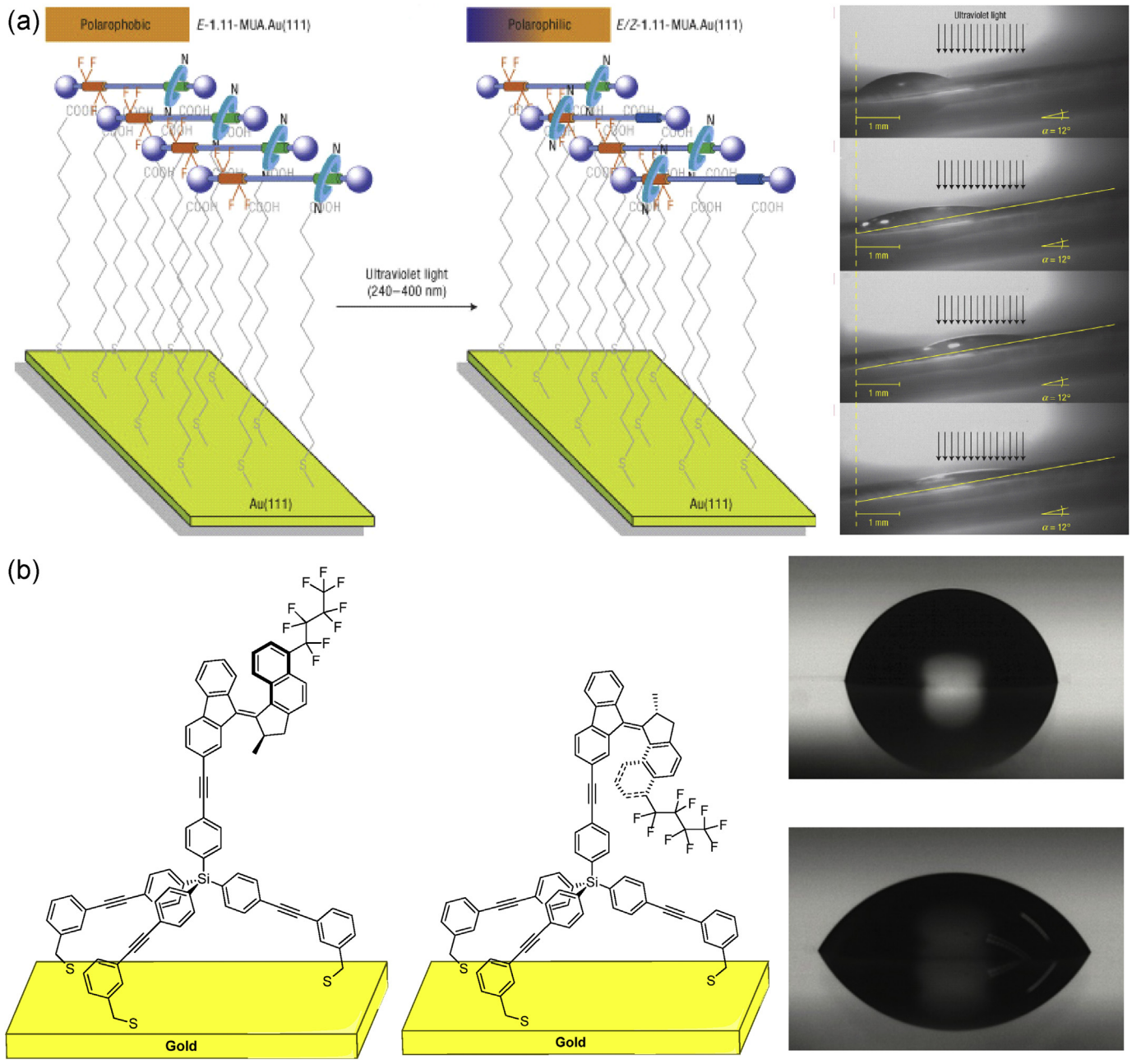

Fig. 3. a) Uphill transport of a diiodomethane droplet. Adapted with permission from Ref.[16], Nature Publishing Group, 2005. b) Modulation of surface wettability with a tripodal anchored molecular motor. Adapted with permission from Ref.[17], American Chemical Society, 2014.

reactions and invert stereoselectivity.

In a different approach, van Esch and co-workers conducted studies towards dissipative molecular self-assembly by their design of a chemically fueled gelation process.[28] Their system was based on dibenzoylcysteine, which at neutral pH does not self-assemble due to repulsive interactions between the negatively charged carboxylate groups (Fig. 5b). The addition of methyl iodide led to the formation of the methyl ester of dibenzoylcysteine, which assembles into fibers and forms a hydrogel. Under neutral conditions, the ester slowly undergoes hydrolysis, resulting in the dissolution of the gel. Upon addition of more fuel (i.e. methyl iodide), the gel is able to reform. Thus only when fuel is present, self-assembly can occur. Although this system is still relatively simple compared to the complexity encountered in natural dissipative self-assemblies, this study constitutes an important step in the development of materials capable of self-healing by continuous regeneration.

In a follow-up paper, the same group used dimethylsulphate as fuel for the dissipative gelation while the overall mechanism for gel formation stayed the same.[29] In this case, it was found that the mechanical behavior of the gel could be tuned using different reaction conditions. By varying the pH and the concentration of the fuel, the stiffness and the storage modulus of the gel could be adjusted. Surprisingly, it was found that the gel state persisted longer than expected based on the concentration of the gelating methyl ester. In order to understand the discrepancy between the molecular and macroscopic behavior, a kinetic analysis of individual gel fibers was performed, which revealed remarkable features. In the regime where fuel is depleted and overall the number of

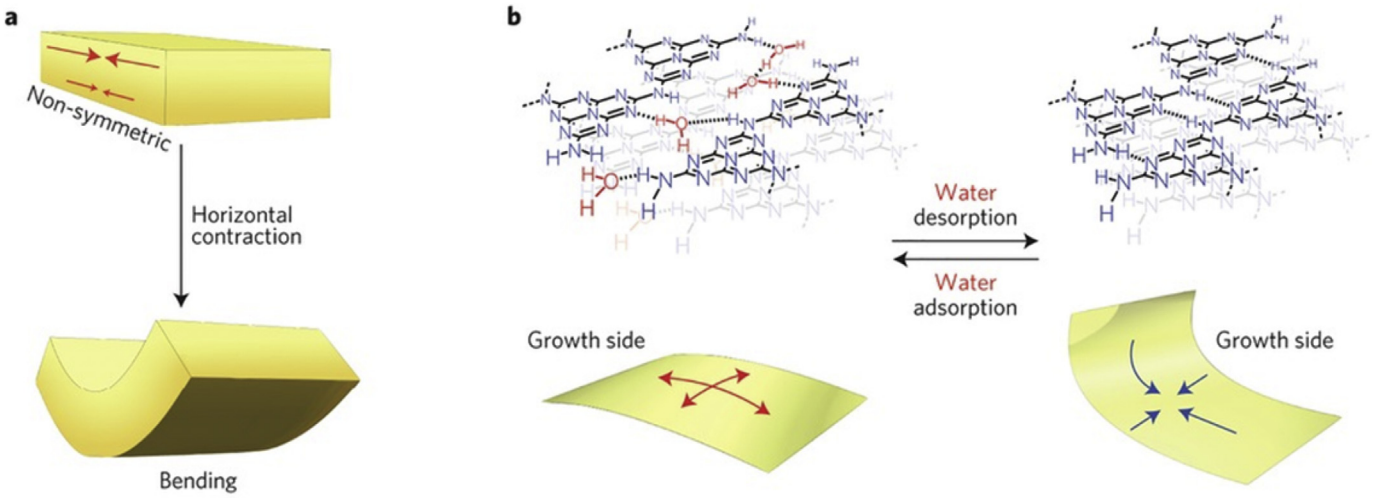

Fig. 4. Proposed mechanism of actuation based on water desorption and adsorption. Adapted with permission from Ref.[22], Nature Publishing Group, 2016.

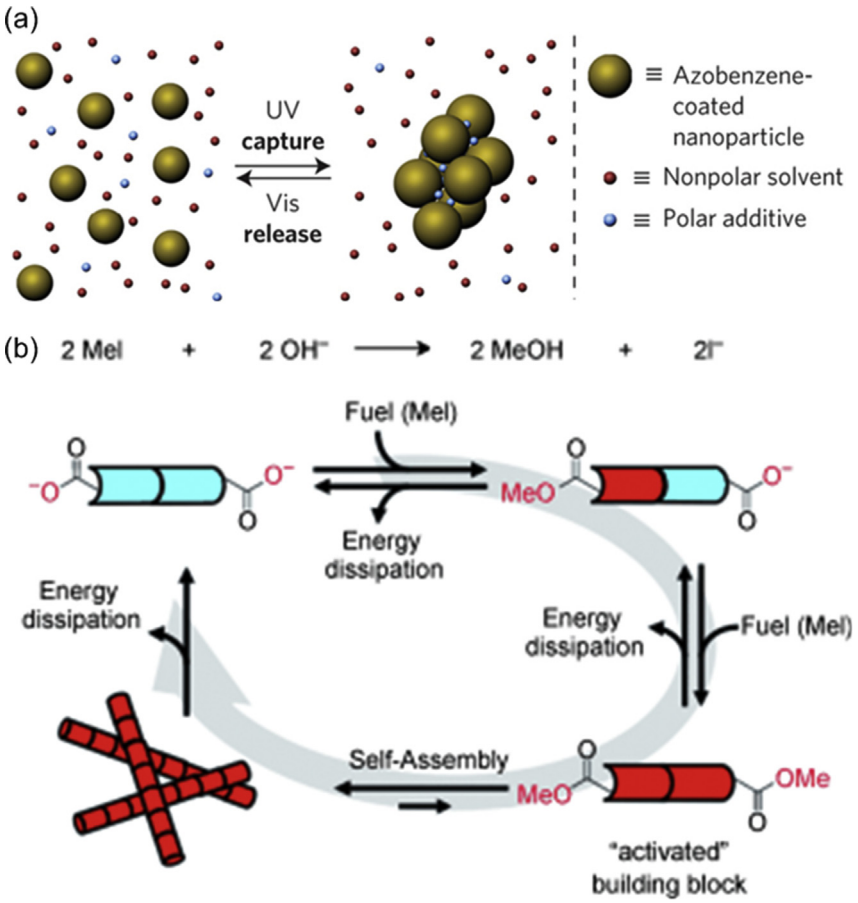

Fig. 5. a) Dissipative self-assembly of gold nanoparticles using light. Adapted with permission from Ref.[27], Nature Publishing Group, 2015. b) Chemically fueled dissipative self-assembly. Adapted with permission from Ref.[28], John Wiley and Sons, 2010.

fibers is decreasing, some growing fibers were still observed. In the regime of gel shrinkage, the fibers did not gradually diminish in size, but displayed a sudden collapse. This behavior is reminiscent of the (de)polymerization dynamics of microtubules. These studies showcase that the macroscopic properties of self-assemblies are principally based on the dynamics at the mesoscopic scale, which ultimately are dictated by the behavior on the molecular scale. Hence, these systems constitute a nice tool to study mesoscopic dynamics, which still remains a largely unexplored field for chemists.

Recently, Prins and co-workers reported on a functional dissipative self-assembly.[30] Their system consisted of a $C_{16}(TACN)Zn^{2+}$

surfactant, which forms vesicles in the presence of ATP. When an enzyme that catalyzes the hydrolysis of ATP is present, vesicle formation becomes a dissipative process. Only when ATP is available the vesicles form as transient structures, with a life-time that is directly related to the rate of hydrolysis of ATP. It was shown that these assemblies can function as nano-reactors, accelerating a nucleophilic aromatic substitution. This work illustrates how the coupling of processes, i.e. the ATP induced formation of C_{16}(TACN) Zn^{2+} vesicles and the hydrolysis of ATP by an enzyme, can be used to achieve dissipative self-assembly.

As illustrated above, azobenzenes are valuable building blocks for light-consuming dissipative self-assemblies since the photogenerated *cis* state is thermodynamically unfavored and relaxes back to the *trans* isomer in the dark. The large change in dipole moment associated with this process can lead to different self-assembly properties. Next to azobenzene switches, molecular motors based on overcrowded alkenes play an important role in the design of dissipative self-assemblies. Also for these compounds, the photogenerated isomers are thermally unstable. Photoisomerization does not cause a large change in dipole, but a pronounced change in structure, which can manifest itself in, for example, a different packing behavior in membranes.[31] The mixing of DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) and an amphiphilic motor in an 1:1 ratio in water resulted in the formation of a nanotubular self-assembly (Fig. 6a). Photoisomerization induces a transition to vesicular assemblies. After heating, the molecular motor reverts to its initial isomer and eventually, the initial tubular structure is reobtained.

The change in chirality of the molecular motor upon photoisomerization and thermal relaxation can also give rise to different

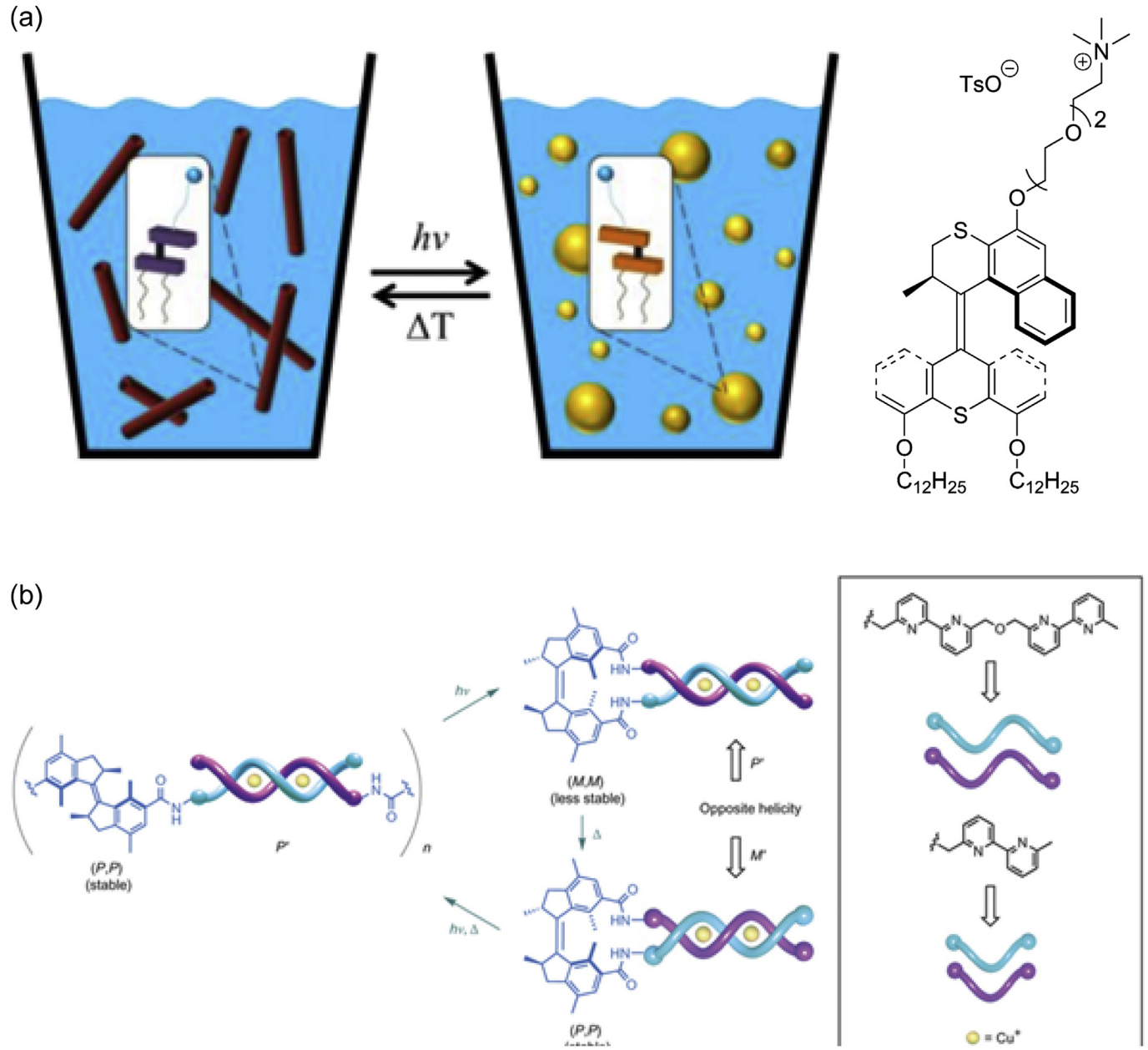

Fig. 6. a) Dissipative formation of vesicles starting from nanotubular structure using a co-assembly of DOPC and an amphiphilic motor. Adapted with permission from Ref.[31], American Chemical Society, 2016. b) Out-of-equilibrium formation of helicate with *P* handedness. Adapted with permission from Ref.[32], Nature Publishing Group, 2016.

self-assembly behavior (Fig. 6b).[32] Inspired by the helicates developed by Lehn,[33] our group developed a molecular motor functionalized with two oligobipyridine units. Upon the addition of Cu^{+}, the *trans* isomer of the motor assembled into an oligomeric array. After photoisomerization to the *cis* isomer, a helicate was obtained, of which the handedness was dictated by the molecular motor. Upon heating, the motor undergoes a thermal relaxation with an concomitant inversion of the helicate's chirality.

4. Self-regulatory and chemical networks

Complex molecular reaction networks govern vital processes in living systems. The cell is a compartment in which the separate components continuously interact and communication with other cells and the external environment is possible through membrane transport.[34] As a result, oscillatory behavior is observed frequently, for example in the circadian clock, in flagella, and in processing neural information.[35] In order to create artificial self-sustaining systems, chemists attempt to mimic the processes of life and take inspiration from oscillations observed in nature. A classic and successful example of a chemical oscillatory reaction is the iconic Belousov-Zhabotinsky reaction.[36]

Oscillations are required to create homeostatic environments. Through a complex network of input, signal processing and feedback loops controlled by chemical and enzymatic reactions, the cell manages to precisely regulate its internal environment. Designing self-regulating reaction networks has drawn attention from researchers in the field of dynamic functional molecular systems, but remains a formidable challenge. In 2012, Aizenberg and co-workers were able to apply these principles to a synthetic material in order to create a self-regulating system.[37]

In their approach (Fig. 7), a surface containing epoxy microstructures is embedded in a hydrogel 'muscle' layer, which is immersed in a liquid bilayer. A catalyst is attached to the top of the microstructures. Below a critical temperature the gel swells, which causes the microstructures to straighten. As a result, the catalysts rise into the top layer of the bilayer, which is rich in reagents that can be converted by the catalyst. The resulting reaction is exothermic, leading to a temperature increase. Above a critical temperature the gel contracts, upon which the microstructures are removed from the top layer and the catalytic reaction no longer takes place. As soon as the temperature then drops below the critical temperature, the same process begins again, i. e. a new oscillation starts.

By variation of the reactions and conditions, the amplitude and frequency of the oscillations can be tuned quite precisely. Temperature fluctuations typically remain below 5 °C, and can be as low as 2.3 °C. The continuous self-regulation of this system provides a real improvement on single directional smart materials. The authors envision that the system can be modified using other stimuli-responsive gels, so that different properties of the material can be regulated.

The creation of self-regulatory networks is not limited to the use of micro/nanostructured materials. There is a general inclination in the chemical sciences to move towards dynamic molecular systems that may be applied under conditions far from equilibrium.[38–40] An important step in this direction was taken by Huck and co-workers, who managed to assemble an oscillating enzymatic system under *in vitro* conditions.[41] This chemical reaction network revolves around the concentration of Trypsin, a small type of serine protease (Fig. 8). Trypsin is generated from its inactive precursor Trypsinogen in an autocatalytic process. In a negative feedback loop, an inhibitor is generated which impedes the formation of Trypsin. This negative feedback loop is split into two parts in order to delay the response with respect to Trypsin generation. As a result, the system can be very precisely engineered by either by modification of the proinhibitor, or alteration of the aminopeptidase involved in the inhibitor activation. Oscillations could be sustained in a large temperature range and over long time periods. Follow-up publications explored the effect of modifying the proinhibitor[42] and coupling the enzyme concentration to hydrogel stiffness, similar to the work of Aizenberg and co-workers discussed above.[43] The regulation of enzyme concentrations in living systems is the result of a very complex network of processes. Huck and co-workers have found a way to translate this feature to an elegant and simple combination of positive and negative feedback loops in a man-made system.[41] However, the use of biomolecules (i.e. Trypsin, Aminopeptidase) is still required for the functioning of these chemical reaction networks. The next step towards fully synthetic systems is to eliminate these biomolecules. The field of chemical networks has recently seen some very important developments in that direction. Pramanik and Aprahamian designed a negative feedback loop to regulate the concentration of zinc(II).[44] Although rudimentary, their system will provide a stepping stone to more advanced fully synthetic oscillating networks. Furthermore, Armao and Lehn studied a constitutional dynamic network under out-of-equilibrium conditions by monitoring reversible imine formation in an evaporating droplet.[45] Additionally, they demonstrated the implementation of nonlinear kinetic behavior into dynamic combinatorial chemistry networks.[46]

While the previous approaches are based on the use of microstructured materials and biomolecules, Whitesides and co-workers envisioned a different approach towards biomimetic oscillating systems. They developed a chemical reaction network solely based on small organic molecules (Fig. 9).[47] Four components are involved in four reactions: amide formation, thiolate-thioester exchange, thiolate-disulfide interchange and conjugate addition, resulting in oscillating thiol concentration in a microfluidic device. At the core of the network is an autocatalytic autoamplification of the thiol, and all reactions proceed without the need for enzyme catalysis. The system can be modulated using different triggers, such as pH, temperature, structural modification, concentration and stirring speed.

Since life on early earth must have originated from small organic molecules, studying these novel networks may uncover the principles that caused chemical evolution towards larger and more complex biomolecular systems. By building further on the principles described here, more advanced and sophisticated reaction

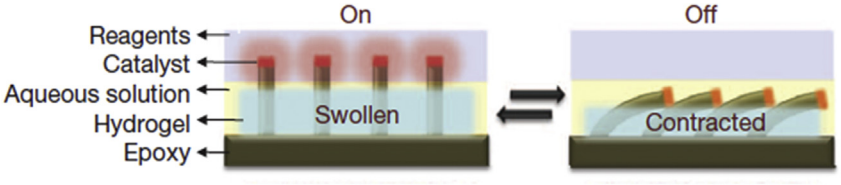

Fig. 7. Schematic overview of the self-regulating materials designed by Aizenberg and co-workers. Adapted with permission from Ref.[37], Nature Publishing Group, 2012.

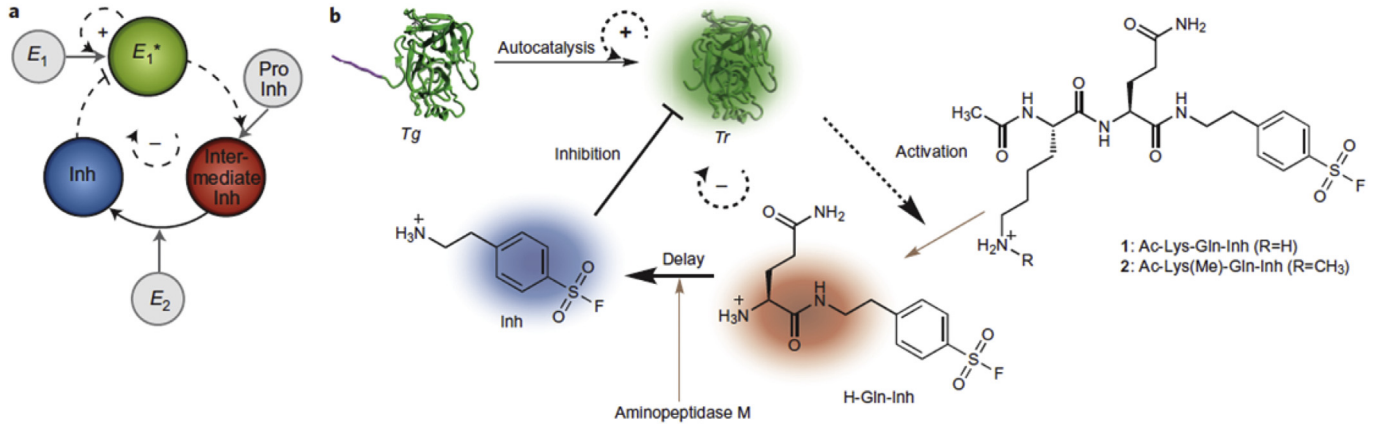

Fig. 8. Oscillating self-regulatory enzyme-catalyzed self-regulatory network, consisting of connected positive and negative feedback loops that regulate the concentration of Trypsin. Adapted with permission from Ref.[41], Nature Publishing Group, 2015.

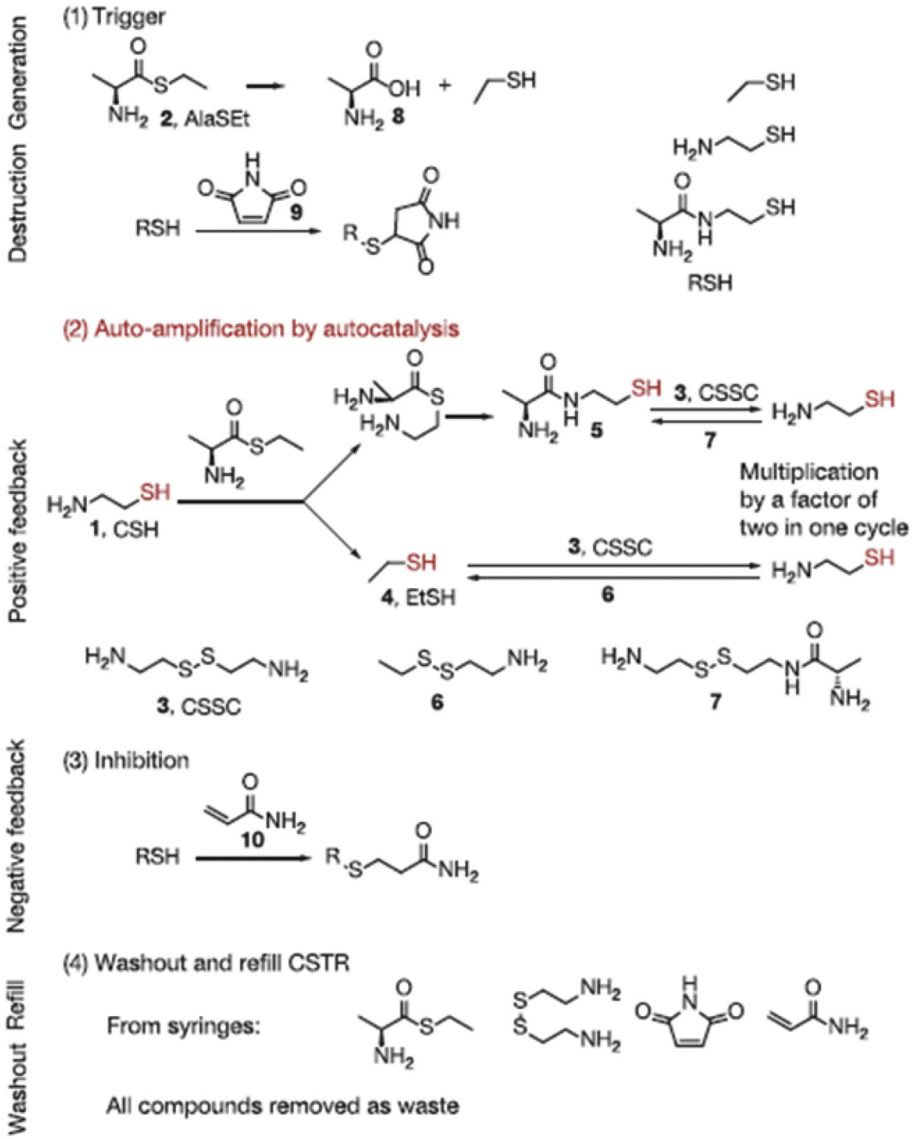

Fig. 9. Autocatalytic self-regulatory network, consisting entirely of small, biologically relevant molecules. Adapted with permission from Ref.[47], Nature Publishing Group, 2016.

networks might be constructed. Especially the small molecule mediated network presented by Whitesides and co-workers[47] may shed light on the origin of life, and will provide a starting point for the development of more elaborated systems.

5. Biohybrid systems

In the medical sciences, there is an ongoing search for highly precise methods to influence and control biological processes. In the past few decades, synthetic chemistry and molecular biology have joined hands in this search. Considerable successes have been achieved with the use of caging methods, wherein a drug is converted to its active form in the body at the location where it is needed.[48] However, with this approach the drug remains present in the body after activation and therefore, a reversible activation strategy is desired. The dynamic features of large biomolecules (e.g. peptides and nucleic acids) are highly advanced compared to synthetic molecules and they are therefore very suitable for use in dynamic functional systems. Due to their programmable nature, these biomacromolecules can be engineered to adopt predictable structures and functions.

In order to elicit a response in a dynamic system, a trigger is required. In dynamic biohybrids, the external trigger is often light, which is both highly tunable and, in contrast to chemical triggers and pH changes, fully orthogonal to the processes in the cell.[49] In this section, we will therefore focus on light-responsive dynamic biohybrids. Nevertheless, it has to be noted that impressive results have also been obtained by the use of other triggers such as pH or temperature. In particular, the dynamic hydrogels developed by Willner and co-workers are worth mentioning.[50,51]

Poly- and oligonucleotides have found application in materials design because of their highly programmable nature.[52] The most successful light-responsive oligonucleotide biohybrids, which we recently reviewed,[53] are based on a system developed by Komiyama and co-workers.[54] In their approach, an azobenzene is tethered to a phosphate backbone linker and inserted into the oligonucleotide (Fig. 10). In the planar *trans* configuration, the photoswitch stacks between neighboring base pairs and stabilizes the double helical structure. The *cis* isomer, on the other hand, is not planar and therefore disturbs the hybridization process. If several azobenzenes are introduced, the two strands can be switched from a double helical structure to two single strands by irradiation with UV light. The reverse process can be induced thermally or through irradiation with visible light. This simple but highly effective method has found a myriad of promising applications, since DNA and RNA are not only vitally important components of a cell, but have also been recognized as enormously versatile building materials.[52] Fundamental cellular processes such as transcription,[55] ligation[56] and DNA cleavage[57] have been successfully controlled in a reversible manner by incorporation of azobenzenes at strategic positions. Towards the development of smart materials, short pieces of complementary DNA modified with azobenzenes have been used as photoreversible glue in order to create larger secondary structures in DNA origami,[58] to modulate hydrogen gel formation[59,60] and to control macrocycle movement in a DNA rotaxane.[61] Finally, the movement of DNA walkers can also be influenced by light taking advantage of azobenzene incorporation.[62,63]

Proteins perform complex tasks that are crucial to life and have therefore not only been of interest to chemists and biologists,[65] but are also considered attractive tools for nanoscientists. Membrane channels regulate the movement of molecules through the bilayer, but with a little engineering they may also be applied in drug delivery. A powerful example of this principle was demonstrated by Feringa and co-workers, who modified a mechanosensitive MscL membrane channel with a spiropyran switch.[66] Light-regulated transport through the channel was achieved, thereby showing effectively that cargo could be released from a liposome by irradiation. SecYEG is a membrane protein channel found in bacteria. Its central pore contains a lateral gate providing an opening to the interior of the membrane. Blocking this gate with a crosslink deactivates the channel, unless the crosslink is longer than 10 Å.[67] This knowledge was utilized by Driessen and co-workers, who installed a crosslinker containing azobenzene in the lateral gate of SecYEG (Fig. 11).[68] For the *trans* isomer, the crosslink is 13 Å long, while for the *cis* isomer the length is reduced to 5–9 Å. With the linker in the *trans* configuration, the channel was shown to work as in the native protein, while after isomerization the translocation of a preprotein through the channel decreased three-to fivefold. The ingenuity of this design is that the activity of a large protein was effectively blocked in a reversible manner without the need to significantly alter the secondary structure.

In a different study that benefits from the use of biological building blocks, Clayden and co-workers have achieved impressive results with the design of synthetic peptide foldamers. These very short peptides adopt a helical structure, which can be influenced using various external triggers. In previous work, the group already demonstrated that the conformational preference of the foldamer could be regulated by switching a photochromic unit, or binding a chiral ligand on one end of the foldamer (Fig. 12).[69,70] In fact, 87% stereocontrol could be recorded over 19 amino acid residues. Recently, the group has demonstrated that this form of information transfer could also be realized through a phospholipid bilayer, both by using a photoswitchable unit[71] and by binding a chiral ligand.[72]

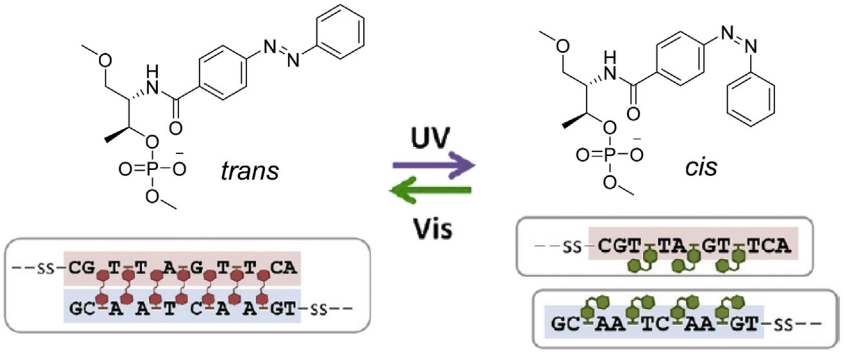

Fig. 10. Photoreversible DNA hybridization through incorporation of azobenzene units. In this example, short complementary single strands of DNA can be used to reversible connect nanoobjects. Adapted with permission from Ref.[64], American Chemical Society, 2012.

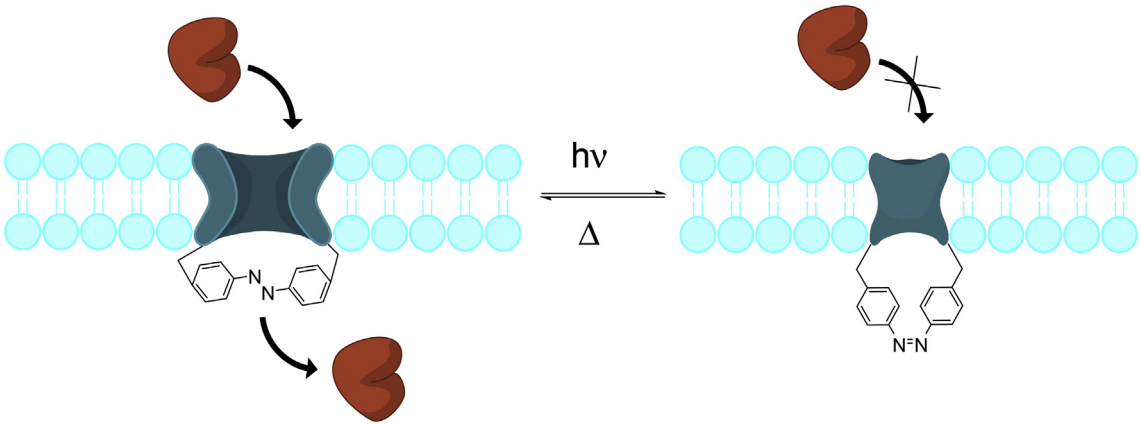

Fig. 11. Photoswitchable SecYEG pore. Preprotein transport is regulated by photoswitching of azobenzene configuration.

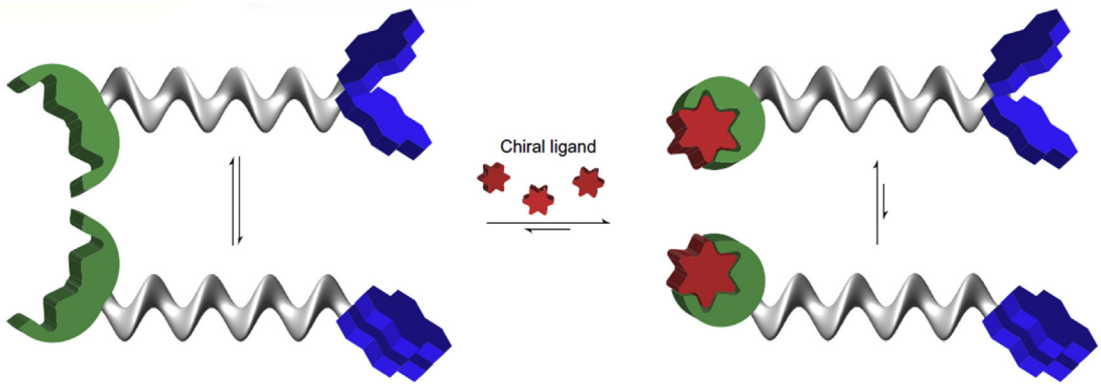

Fig. 12. Reversible binding of chiral ligand leads to a change in equilibrium distribution. Adapted with permission from Ref.[72], Nature Publishing Group, 2017.

As such, they prove that their design of a simplified analog for membrane spanning proteins may in fact be operated in the membrane. Although much simpler than a real protein, these foldamers bear a striking resemblance to G-protein coupled receptors found in nature, and may be applied for information transfer in the design of artificial cells.

Photopharmacology relies on the incorporation of a photoswitch into a drug in order to regulate its activity by irradiation and this relatively new field has been well reviewed elsewhere.[73–75] Usually, this approach involves small bioactive molecules such as antibiotics[76] and chemotherapy agents,[77] but some examples involve peptides or proteins. Trauner, Isacoff and coworkers developed a photoswitchable linker that could regulate the opening and closing of a Light-gated Ionotropic Glutamate Receptor (LiGluR), a receptor involved in vision.[78,79] Using adeno-associated viral vectors, the LiGluR was expressed in retinal ganglion cells of blind mice.[80] As a result, light sensitivity is restored, generating response in the primary visual cortex, pupillary reflexes and natural light-avoidance behavior. These results are a striking example of the effectiveness of light-responsive unit incorporation *in vivo*, and clearly demonstrate the advantages of a dynamic approach.

Controlling the structure of biomolecules in a responsive, dynamic way thus can lead to advanced control over biological function. Considerable successes have been achieved using biohybrid systems of peptides or oligonucleotides and photoresponsive units. In particular the results obtained by DNA/RNA based biohybrids show that such methods are not limited to biological systems, but may also be used in materials design or nanotechnology.

6. Conclusions

In summary, unique approaches towards the construction of dynamic functional molecular systems have been discussed, thereby focusing on the design principles. Areas that are covered range from responsive materials and far-from-equilibrium self-assemblies to self-regulatory networks and biohybrid systems. A wide variety of chemical and physical stimuli have been successfully applied for triggering responsive functions, such as pH, ion binding, chemical oxidation/reduction and light. The latter being used mainly to address photoswitchable units that are embedded within soft materials and biomolecules or anchored onto surfaces. Where azobenzenes are most commonly used in this regard, the unique features of light-driven molecular motors (e.g. continuous rotary motion, multi-state chiroptical switching) offer endless and exciting new opportunities.

Chemical stimuli-responsive systems, on the other hand, enable the implementation of feedback loops and self-regulation, akin to the processes that take place in biology. Coupling of chemical and catalytic conversions to dynamic functions requires a profound understanding of the chemical kinetics and equilibria involved. Recent progress in the field as discussed here provides an excellent basis to build on further and, although great challenges lie ahead of us, the prospects for dynamic molecular systems are particularly bright.

Acknowledgements

Financial support from The Netherlands Organisation for

Scientific Research (NWO-CW, Veni grant no. 722.014.006 to S.J.W.), the Ministry of Education, Culture and Science (Gravitation program no. 024.001.035), and the European Research Council (Advanced Investigator Grant, no. 694345 to B.L.F) is gratefully acknowledged.

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Anouk S. Lubbe studied chemistry at the University of Groningen, where she received her MSc in 2012 after working with Prof. Ben L. Feringa on molecular motors. In 2012, she worked with Prof. Jean-Marie Lehn at the University of Strasbourg on dynamic covalent metallosupramolecular grids. She is currently pursuing her PhD under the supervision of Prof. Ben L. Feringa and Dr. Wiktor Szymanski. Her research focuses on the application of molecular motors in biological settings and the study of molecular motors in new media.

Thomas van Leeuwen was born in 1988 in Emmen (The Netherlands). He studied chemistry at the University of Groningen, where he received his MSc in 2012 after working with prof. B. L. Feringa on the application of molecular motors for photoswitchable catalysis. After an internship in the group of prof. T. Bach at the Technical University of Munich, he started his PhD under the supervision of Prof. B. L. Feringa, working on the synthesis and application of molecular motors.

Sander J. Wezenberg studied chemistry at the University of Nijmegen where he carried out his Master's research in the group of Prof. Roeland Nolte. He then moved to Tarragona for his PhD studies in the field of supramolecular chemistry with Prof. Arjan Kleij at the Institute of Chemical Research of Catalonia (ICIQ). During this period he spent three months as a visiting researcher in the group of Prof. Joseph Hupp at Northwestern University. After receiving his PhD in 2011, he joined the group of Prof. François Diederich at ETH Zurich as a postdoctoral fellow. He came to Groningen in 2013 to work with Prof. Ben Feringa at the Stratingh Institute for Chemistry where he was later appointed Assistant Professor. His main research interests are in the areas of anion binding and transport, molecular switches, and self-assembled materials.